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نموذج رقم (١٨)
اقرار والتزام بقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير

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عنوان الرسالة / الاطروحة
Development of Biorelevant Dissolution Testing conditions for Fat-Based Progesterone Pessaries and their Potential Application for in vitro-in vivo correlation (IVIVC)

أعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير والدكتوراة عندما قمت شخصيا" باعداد رسالتي / اطروحتي ، وذلك بما ينسجم مع الأمانة العلمية المتعارف عليها في كتابة الرسائل والأطاريح العلمية. كما أنني أعلن بأن رسالتي /اطروحتي هذه غير منقولة أو مستلة من رسائل أو أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فإنني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

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DEVELOPMENT OF BIORELEVANT DISSOLUTION TESTING CONDITIONS FOR FAT-BASED PROGESTERONE PESSARIES AND THEIR POTENTIAL APPLICATION FOR IN VITRO-IN VIVO CORRELATION (IVIVC)

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This Thesis Was Submitted In Partial Fulfillment Of The Requrement For The Master's Degree Of Science In Pharmaceutical Sciences

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COMMITTEE DECISION

This Thesis/Dissertation (Development of Biorelevant Dissolution Testing Conditions for Fat-based Progesterone Pessaries and their Potential Application for in vitro-in vivo correlation (IVIVC)) was Successfully Defended and Approved on 8/8/2011.

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List of Abbreviations

AIC	Akaike Information Criterion
ANDA	Abbreviated New Drug Application
AUC_0^{∞}	Area under the plasma concentration versus time curve from time zero to time infinity
AUC_{last}	Area under the plasma concentration versus time curve from time zero to last time point with measurable concentration
^0C	Degrees Celsius
Cmax	Maximum plasma drug concentration
f_1	Difference factor
f_2	Similarity factor
HPBCD	Hydroxypropyl-beta-cyclodextrin
FDA	Food and Drug Administration
HPLC	High Performance Liquid Chromatography
IVIVC	*In vitro-in vivo* correlation
k_a	Absorption rate constant
k_e	Elimination rate constant
MRT	Mean residence time
NDA	New Drug Application
PK	Pharmacokinetic
PRU	Pharmaceutical Research Unit
rpm	Rounds per minute
RSD	Relative standard deviation
SD	Standard deviation
SLS	Sodium lauryl sulfate
Tmax	Time needed to reach peak plasma concentration
UIR	Unit impulse response
USP	United states Pharmacopoeia

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Abstract

The aim of this work was to develop a reliable dissolution testing procedure for fat-based progesterone pessaries to provide a reliable quality control test for them, and to evaluate the potential of this method to develop an *in vitro*-*in vivo* correlation (IVIVC). Since progesterone is very poorly soluble in water, hydroxypropyl-beta-cyclodextrin (HPBCD) and sodium lauryl sulfate (SLS) were used as solubilizers to provide sink conditions for the dissolution process; however, the required SLS concentrations were too high resulting in a too harsh environment for the release process, so HPBCD was the chosen solubilizer. 10% w/w HPBCD solution was used as a dissolution medium and was evaluated in different setups. The use of 900 ml of the 10% w/w HPBCD solution in type II United States Pharmacopoeia (USP) dissolution testing apparatus at 37^{0}C and 100 rpm gave acceptable dissolution results. Dissolution profiles generated for three products were used to construct Levy plots using *in vivo* data obtained from bioequivalence studies performed on these products. The resulting Levy plots gave high correlation coefficients greater than 0.95 indicating a good potential for IVIVC, but *in vitro* and *in vivo* data for products with significantly different release rates are needed to validate an IVIVC and ensure the discriminating power of the dissolution testing method.

1-Introduction

1.1-Dissolution

Dissolution rate may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition (Abdou, 1989). Since, in order to be absorbed, a drug must be present in solution liberated from the dosage form in which it was administered, its dissolution is an important step of its absorption especially for poorly soluble drugs where, because of the slow dissolution rate, the absorption process is dissolution rate-limited. In addition to dissolution, the release of a drug from its dosage form may involve other processes like, for example, partitioning of drug dissolved in a lipid matrix from the lipid phase to the aqueous medium into which release occurs.

Since the release of a drug from its formulation is an important determinant of its bioavailability, particularly when it is the rate-limiting step of the absorption process, it is considered as the most important measure for assessing formulation performance. Therefore, an *in vitro* testing procedure to evaluate drug release is needed. Hence came a multitude of "dissolution testing" procedures that have been reported in the literature and some of them have become official tests employed by pharmacopoeias.

Therefore, dissolution testing is a very important quality control test employed by the pharmaceutical industry (Siewert *et al.*, 2003). It is used routinely in both Quality Control (QC) and Research and Development (R&D) departments of a pharmaceutical firm, with its focus being on ensuring batch to batch consistency and

detecting manufacturing deviations in a QC department, and on providing predictive estimates for the *in vivo* performance of a drug product in an R&D department (Azarmi *et al.*, 2007). According to Food and Drug Administration (FDA) regulations, a dissolution test should be included in both a New Drug Application (NDA) and an Abbreviated New Drug Application (ANDA) (FDA, 2000). When developing generic products, comparative dissolution testing of the innovator and the generic products should be performed and submitted to the regulatory authorities as a part of the proof of the equivalence of the two products; when a dissolution testing method is available in the United States Pharmacopoeia (USP), it should be used, if not, then the procedure listed by the innovator in the NDA should be followed, but this is not always available to public, and, in this case, a comparative dissolution method should be developed in house (FDA, 2000).

The two apparatuses that are most commonly used for dissolution testing are the Basket and Paddle apparatuses (USP type I and USP type II dissolution testing apparatuses respectively). Both are stirred-vessel type apparatuses employing the same type of vessel filled with a dissolution medium of controlled volume and temperature with the paddle apparatus involving the placement of the tested dosage form in the vessel and agitating the medium by a rotating paddle, and the basket apparatus involving the placement of the tested dosage form in a small basket formed from a screen that is immersed in the dissolution medium and rotated at a given speed (Alderborn, 2007). Other less common compendial apparatuses include the USP type III apparatus called the reciprocating cylinder apparatus, and the USP type IV apparatus called the flow through apparatus.

1.2- Biorelevant Dissolution Testing

In order for the dissolution method to be a reliable indicator of the product performance, its results should reflect as closely as possible the *in vivo* performance of the product. Hence came the concept of biorelevant dissolution testing.

A biorelevant dissolution test is capable of predicting *in vivo* bioavailability by means of an *in vitro-in vivo* correlation (IVIVC), and so it is capable of significantly reducing the time and costs associated with *in vivo* pharmacokinetic and clinical studies during drug product development (Klein, 2010).

IVIVC's fall into the following categories (FDA, 1997):

A. Level A IVIVC:

Correlates the fraction of drug absorbed to the fraction of drug dissolved. A correlation of this type is generally linear and represents a point-to-point relationship.

B. Level B IVIVC:

Uses the principles of statistical moment analysis. The mean *in vitro* dissolution time is compared either to the mean residence time or to the mean *in vivo* dissolution time. Even though it uses all of the *in vitro* and *in vivo* data, it is not considered to be a point-to-point correlation since similar mean residence time values can be provided by different plasma concentration profiles.

C. Level C IVIVC:

Is a single point relationship between a dissolution parameter (like the %release in a certain time or time required to achieve a certain %release) and a pharmacokinetic parameter (like AUC, Cmax and tmax).

D. Multiple level C IVIVC:

Relates one or more pharmacokinetic parameters to several points of the dissolution profile. It can be as useful as a Level A IVIVC; however, if a multiple Level C IVIVC is obtained, it means that obtaining a Level A IVIVC is likely and it is preferred.

In order to develop a biorelevant dissolution test, consideration should be given for the physicochemical properties of the drug, its formulation and the physiologic factors affecting its absorption (Klein, 2010). Many publications deal with biorelevant dissolution testing and its potential applications. For example, biorelevant dissolution enabled the establishment of a Level A IVIVC for oral modified release pellet dosage form of diclofenac sodium, and, to correctly, predict the effect of food on the *in vivo* performance of this product (Jantratid et al., 2009). Another biorelevant dissolution test was found to be able to correlate well to the *in vivo* performance of diclofenac sodium extended release tablets, and contrary to conventional dissolution testing, was able to account for the appearance of multiple peaks in the pharmacokinetic profile obtained following the administration of the product (Garbacz et al., 2008).

Biorelevant dissolution testing helped in concluding that precipitation of nifedipine in the stomach was responsible for the delay in absorption following its administration in the form of soft-gelatin capsules (Thelen et al., 2010). However, in general, most of the research on biorelevant dissolution testing has been restricted to oral formulations and only few publications deal with the subject concerning non-oral dosage forms.

1.3- Vaginal Drug Delivery

The vagina is an about 10 cm long tubular fibromuscular canal lined with a mucous membrane that extends from the vulva (its opening to the exterior of the body) to the uterine cervix (Tortora and Derrickson, 2005). Drugs are administered to the vagina mainly for the achievement of local effects like treating microbial infections of the vagina; however, it has been used for the systemic administration of certain drugs like oestrogens, prostaglandin analogues and progesterone (Tukker, 2007). The vaginal wall is, indeed, suitable for the systemic absorption of drugs due to its rich blood supply, and, as in the case of progesterone, higher bioavailability can be achieved by using this route compared to the oral route due to the avoidance of first-pass metabolism (Tukker, 2007). Drugs can be administered into the vagina as suppositories (pessaries), tablets, capsules, solutions, sprays, creams, ointments and foams (Winfield, 2005).

Suppositories can be made from fatty bases or water-soluble bases (Tukker, 2007). When the suppository base is water soluble, the drug is released after the suppository base dissolves, while in case of fatty suppositories, drug release occurs following the melting and spreading of the suppository base as the drug particles sediment out of the molten fat and dissolve at the interface between the molten fat and vaginal fluid in case the drug is suspended in the suppository base, or the drug simply partitions between the molten base and the vaginal fluid in case it is dissolved in the base (Abdou, 1989).

1.4- Progesterone

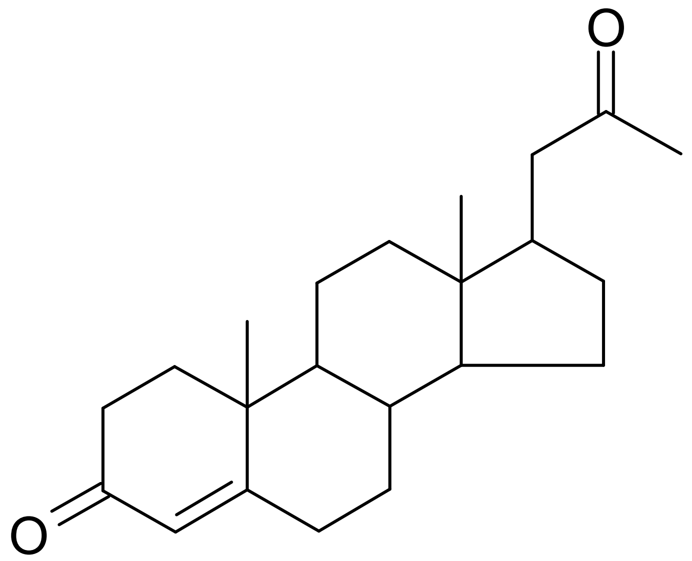


Figure 1. Structure of progesterone

Progesterone is the most important natural progestetational hormone in humans, and is synthesized mainly in the ovaries by the corpus luteum during the luteal phase of the menstrual cycle and by the placenta during pregnancy (Chrousos, 2004). It is responsible for the alveololobular development of the secretory apparatus in the breast and causes the maturation and the secretory changes observed in the endometrium following implantation (Chrousos, 2004). During pregnancy, it maintains the uterine lining and ensures that the cervix remains closed, and, in addition, it promotes the development of mammary glands and ensures that uterine smooth muscles remain relaxed (Tortora and Derrickson, 2005).

Progesterone is used for the treatment of secondary amenorrhea, prevention of endometrial hyperplasia in post-menopausal women receiving oestrogens and abnormal uterine bleeding due to hormonal imbalances, and as a part of assisted reproductive technology (ART) for infertile women with progesterone deficiency.

Progesterone pharmacokinetics are characterized by extensive plasma protein binding and rapid elimination (Chrousos, 2004). It can be administered orally, intramuscularly and intravaginally. Progesterone has been administered in the form of pessaries (vaginal suppositories) for some years and better results have been obtained with this route compared to the oral route due to the avoidance of the extensive first-pass metabolism encountered following oral administration and the extensive blood supply for the vaginal wall (Tukker, 2007).

As for its physicochemical characteristics, it is a neutral steroidal molecule without any ionizable groups. Progesterone exhibits very low solubility in water of 3.4 mg/L (Melisoglu-Bilensoy *et al.*, 2006). This poses a challenge to the development of a biorelevant dissolution test for progesterone pessaries which are available as 400 mg fat-based pessaries in the Jordanian market since, in order to obtain sink conditions mimicking the removal of the absorbed progesterone by the bloodstream, the concentration of the drug released to the dissolution media should not exceed 10-20% of the saturation solubility at any time during the dissolution test (British Pharmacopoeia, 2007).The problem can be solved either by using a flow-through apparatus or by incorporating a suitable solubilizing agent in the dissolution medium.

In general, it is difficult to recommend a dissolution testing method for suppositories due to the variety of formulation characteristics of this type of dosage forms; however, a modified flow-through apparatus with a specific dual chamber for the suppository may be a good idea to start with when developing a dissolution method for lipophilic suppositories (Siewert *et al.*, 2003).

In this work, we aimed to develop a reliable dissolution method for progesterone pessaries. Since we could not obtain the modified flow through apparatus, we could not use it. However, we used hydroxypropyl-β-cyclodextrin (HPBCD) to increase the solubility of progesterone in the dissolution medium by forming an inclusion complex with the released progesterone thus enabling the development of a biorelevant dissolution test for fat-based progesterone pessaries.

2-Experimental part

2.1-Equipment

An Erweka DT600 dissolution tester was used for dissolution testing. The chromatographic system consisted of a Shimadzu SIL-10ADVP autosampler, LC-10ADVP pump, DGE-14A degasser, SCL-10ADVP controller and SPD-M10A VP Diode Array detector. The separation was performed using a C18 analytical column (250 mm × 4.6 mm, 5 µm; Thermo Hypersil, UK). Data recording was carried out using Shimadzu Class VP System. A Knauer chromatograph equipped with a Smartline isocratic pump 1000, a Smartline UV detector 2500 and a 20 µL injection loop was also used. A LabTech (South Korea) shaking incubator was used for the phase solubility studies.

2.2-Materials

Three registered progesterone pessary products (A, B and C), each manufactured by a different manufacturer were used. Reference (A) pessaries were obtained from the market while B and C pessaries were obtained from the Pharmaceutical Research Unit (PRU, Amman, Jordan). In addition to progesterone, product A contained vegetable fat the exact identity of which was not disclosed by the manufacturer, while products B and C contained Suppocire BS2. Progesterone was obtained from Joriver, spironolactone from Sigma (China), and hydroxypropyl-beta-cyclodextrin (HPBCD) from Hebei Smart Chemicals (China). High performance liquid chromatography (HPLC) grade methanol and acetonitrile were obtained from Tedia Company (OH, USA). Sodium lauryl sulfate (SLS) was obtained from Jordanian Pharmaceutical Manufacturing Company (JPM).

2.3- HPLC method for progesterone analysis

A mobile phase consisting of water, methanol and acetonitrile in the volume ratio 30:35:35 was used. To an aliquot of the tested solution, spironolactone solution, in 70% methanol, used as the internal standard, was added in an amount sufficient to obtain a spironolactone concentration of 20 µg/ml, and a progesterone concentration within the validated range, following the dilution step. After doing the dilution with 70% methanol, the resulting solutions were chromatographed using an injection volume of 20 µL. The mobile phase flow rate was maintained at 1.0 ml/min and the detection wavelength was 240 nm.

A calibration curve was constructed from five progesterone solutions covering a range of concentrations from 3 to 60 µg/ml, with each concentration being injected in triplicate, to evaluate linearity, accuracy and precision. The selectivity of the method was evaluated by injecting blank dissolution medium treated in the same way as the sample minus the addition of the internal standard.

The method was also validated on a Knauer chromatograph over a range of progesterone concentrations ranging from 4 to 60 µg/ml, using five progesterone solutions with different concentrations, and, also, with each concentration being injected in triplicate.

2.4- Phase solubility studies

Excess solid progesterone was added to 25 ml of 2, 3.85, 7.41, 16.67 and 28.57% w/w solutions of HPBCD, and 0.5, 1.0, 1.5, 2, 2.5 and 3.0% solutions of sodium lauryl sulphate (SLS). The mixtures were shaken for 48 hours in an incubator at 37^0C.

Aliquots of the mixtures were then filtered using 0.45µm nylon filters, and the filtrate was analyzed using HPLC.

2.5- *In vitro* dissolution studies

The dissolution test was performed in triplicate, with each pessary being placed in a USP type II apparatus dissolution vessel containing 900 ml of 10% w/w HPBCD at 37^0C, and the paddle was rotated at a speed of 100 rounds per minute (rpm). 5 ml samples were taken manually with complete replacement with fresh dissolution media. The samples were appropriately filtered through 0.45 micron cellulose acetate filters, then, to a portion of each sample, the internal standard was added, dilution was performed in 70% methanol and the resulting solution was chromatographed. The sampling times were 15, 20, 25, 30, 35, 40, 60 and 90 minutes. Performing the test at 50 and 75 rpm was also attempted. USP type I (basket) method was also attempted using the same medium, 100 rpm and a Vankel VK700 dissolution testing apparatus at 37^0C with an autosampler. In addition, a release test using the Vankel VK700 equipment with the same volume of the same medium, temperature, rotation speed and sampling methodology was performed, but this time the suppository was placed in a dialysis cell constructed from a dissolution basket enclosed by a cellulose acetate dialysis membrane with a molecular weight cut-off of 14 kDa. This dialysis cell was placed in the dissolution medium in the vessel, and the medium was agitated with the paddle at 100 rpm. Initially each setup was attempted for the reference product (product A), and, then, the setup that provided acceptable results was repeated for the two other products.

2.6- *In vivo* pharmacokinetic studies

These were performed at the PRU as two bioequivalence studies, one comparing product B (test) to product A (reference), and the other comparing product C (test) to product A (reference). Two bioequivalence studies were performed: one comparing product B to product A, and the other comparing product C to product A. Both studies were of the two-period crossover design with the first study involving 34 volunteers and the second one involving 32 volunteers. The data were analyzed using WinNonlin 5.2 software. All the volunteers in both studies were healthy post-menopausal females.

3-Results and discussion

3.1- HPLC analysis

The analytical method was found to be linear over the progesterone concentration range 3-60 µg/ml.

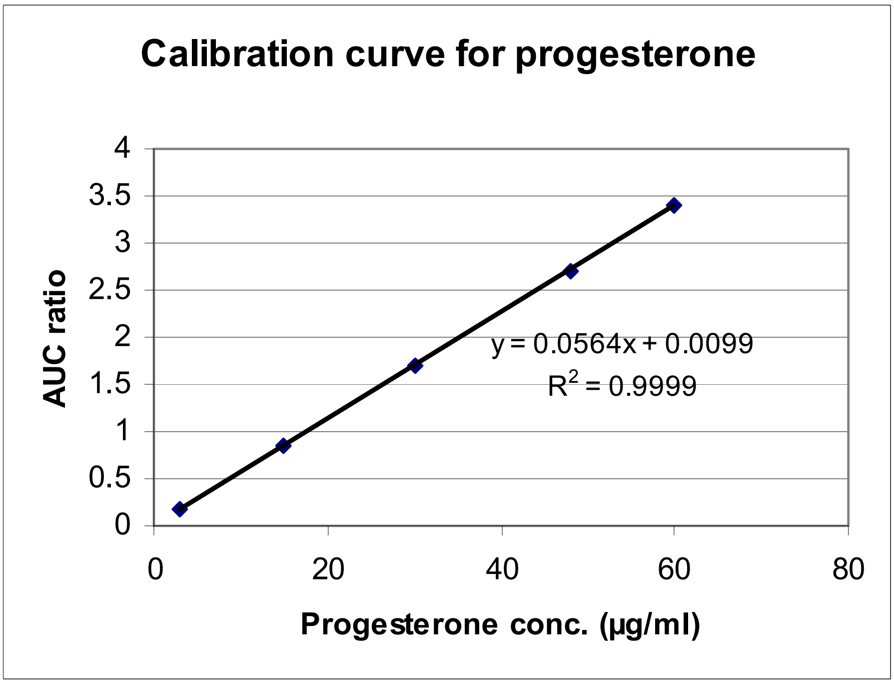


Figure 2. Calibration curve for progesterone HPLC analysis

The accuracy and precision of the method are presented in the following table:

Table 1. Precision and accuracy data for the HPLC method used for progesterone analysis

Progesterone conc. (µg/ml)	Progesterone conc. determined (µg/ml)	%RSD	%Accuracy
3	3.09	1.58	3.00
15	14.66	0.29	2.27
30	30.15	0.75	0.50
48	47.73	0.091	0.56
60	60.21	0.15	0.35

The data in the above table show that the method is both accurate and precise, and the high r^2 value exhibited by the calibration curve in figure 2 shows that good linearity is exhibited by the method.

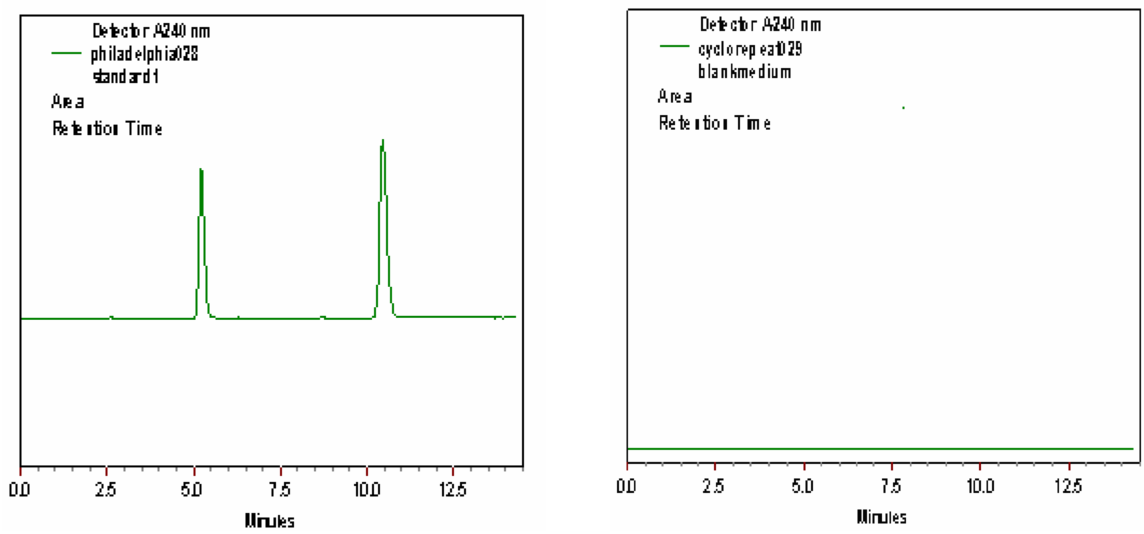


Figure 3. Chromatograms showing the peaks of the analyte and the internal standard (left), and the result of injecting blank dissolution medium (right).

The fact that the method is selective is shown by the chromatograms in figure 3, with the left-hand side chromatogram showing the peaks of progesterone (with a retention time of 10.73±0.050 min) and spironolactone (with a retention time of (5.47±0.048 min), and the right-hand side chromatogram showing the result of injecting blank dissolution medium treated in the same way as a sample.

The method was also validated for progesterone concentrations ranging from 4 to 60 µg/ml on a Knauer instrument as shown in table 2 and figure 4.

Table 2. Validation results of the analytical method on the Knauer chromatograph

Progesterone conc. (µg/ml)	Progesterone conc. determined (µg/ml)	%RSD	% Accuracy
4	3.93	0.57	1.75
10	9.84	1.99	1.6
30	30.51	1.15	1.7
50	49.96	0.73	0.08
60	59.79	1.43	0.35

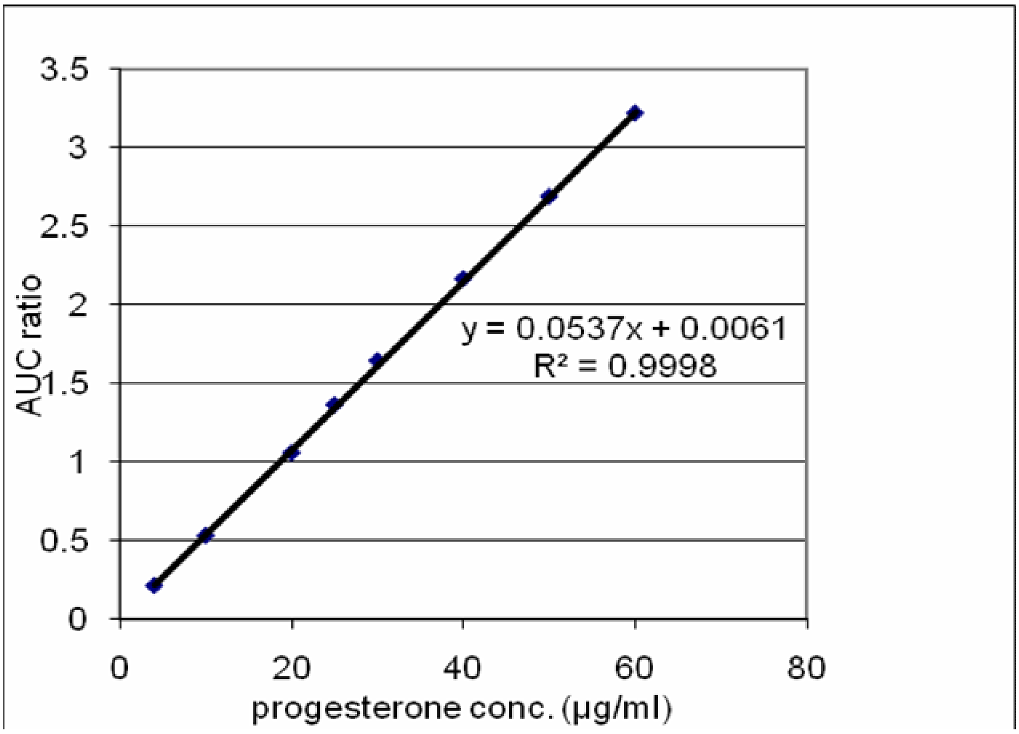


Figure 4. Calibration curve for progesterone analysis on the Knauer chromatograph

3.2- Phase solubility studies results

Both SLS and HPBCD were found to be capable of raising the progesterone solubility to values sufficient for attaining sink conditions during dissolution of 400 mg progesterone pessaries as shown by the following tables:

Table 3. Phase solubility study results for the solubilization of progesterone by SLS

SLS concentration (%w/v)	Progesterone solubility (mg/ml)	Volume saturated by 400 mg of progesterone (ml)
0.50	0.726	551
1.00	1.55	258
1.50	2.72	147
2.00	3.57	112
2.50	4.55	88
3.00	5.34	75

Table 4. Phase solubility study results for the solubilization of progesterone by HPBCD

HPBCD concentration (%w/w)	Progesterone solubility (mg/ml)	Volume saturated by 400 mg of progesterone (ml)
2.00	0.956	418
3.85	2.066	194
7.41	5.064	79
16.67	10.706	37
28.57	20.955	19

3.3- *In vitro* dissolution results

When 3% SLS was used, the dissolution conditions were too harsh resulting in a dispersion of the molten suppository that could not be filtered by the available means (passing the dispersion through 70 micron ultra-high molecular weight polyethylene cannula filters, 0.45 micron cellulose acetate membrane filters, 0.45 micron nylon membrane filters, and even through two successive nylon and cellulose acetate 0.45 micron membrane filters was attempted without any noticeable effect on the clarity of the filtrate). HPBCD did not disperse the molten base giving relatively mild dissolution conditions. The basket method gave excessively slow release (50% at 72 hours as shown in table) even though the %relative standard deviation (%RSD) values obtained were acceptable, and the method using dialysis membranes gave 12% release at 72 hours. Using rpm values of 50 and 75 rpm for the paddle method gave %RSD values greater than 20% at more than 30% release and so were considered unacceptable. The paddle method using 100 rpm gave the most acceptable results, and was performed for the three products, with the results being shown in table 5 and figure 5.

The dissolution profiles obtained using the paddle method at 100 rpm were fitted using the zero order, first order, Higuchi, Hixson-Crowell's cube root law and Korsmeyer-Peppas release equations(Hixson and Crowell, 1931; Higuchi, 1963; Gibaldi and Feldman, 1967; Korsmeyer et al., 1983), as follows:

Zero order kinetics : $F_t = k_0 t$ $_{(1)}$

where Ft represents the fraction of drug released in time t and k_0 the apparent release rate constant or zero order release rate constant.

Table 5. ***In vitro*** **release test results for the three products using the paddle method at 100 rpm**

time (min)	Product								
	A			B			C		
	%release*	SD	%RSD	%release*	SD	%RSD	%release*	SD	%RSD
15	19.5	3.8	19.5	9.2	1.5	16.3	9.1	6.8	74.7
20	29.4	4	13.6	18.2	4.8	26.4	28.0	7.4	26.4
25	37.6	4.6	12.2	33.5	4.8	14.3	40.8	6.9	16.9
30	43.9	2.7	6.2	49.7	5	10.1	52.8	8.2	15.5
35	52.6	2.4	4.6	62.4	4.7	7.5	62.6	8.8	14.1
40	60.0	1.7	2.8	71.7	4.8	6.7	72.3	8.0	11.1
60	79.7	2.8	3.5	84.5	4.6	5.4	86.6	2.9	3.3
90	87.9	2.8	3.2	91.0	0.9	1.0	91.7	0.8	0.9

***arithmetic mean**

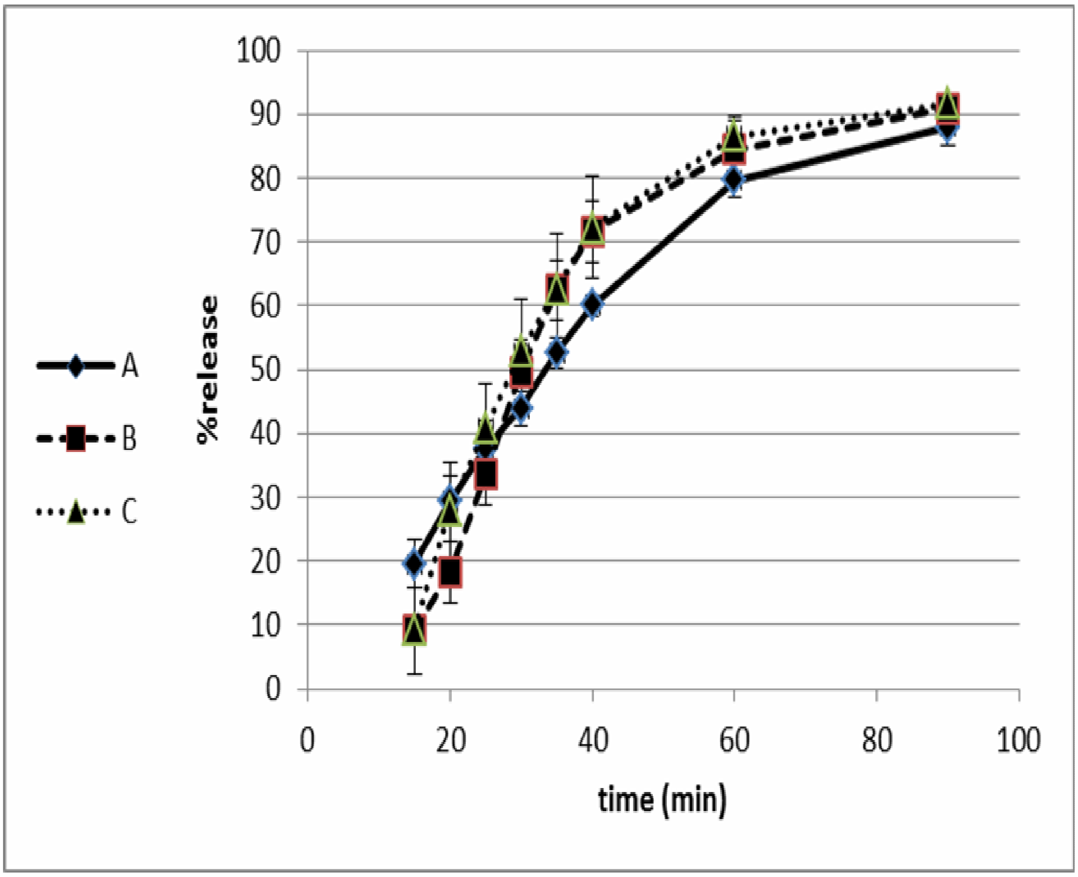


Figure 5. Mean ***in vitro*** **release test results for the three products with vertical error bars showing one standard deviation on each side**

Table 6. ***In vitro*** **release test results for the three product A using the basket method at 100 rpm**

time (h)	%release*	SD	%RSD
1	8.3	1.1	13.3
2	9.3	1.1	11.8
4	12.1	0.9	7.5
6	14.2	0.8	5.6
9	16.9	1.5	8.9
12	19.2	0.8	4.2
16	22.3	1.1	4.9
24	27.9	1.8	6.5
48	39.9	1.1	2.7
72	50.1	0.6	1.2

***arithmetic mean**

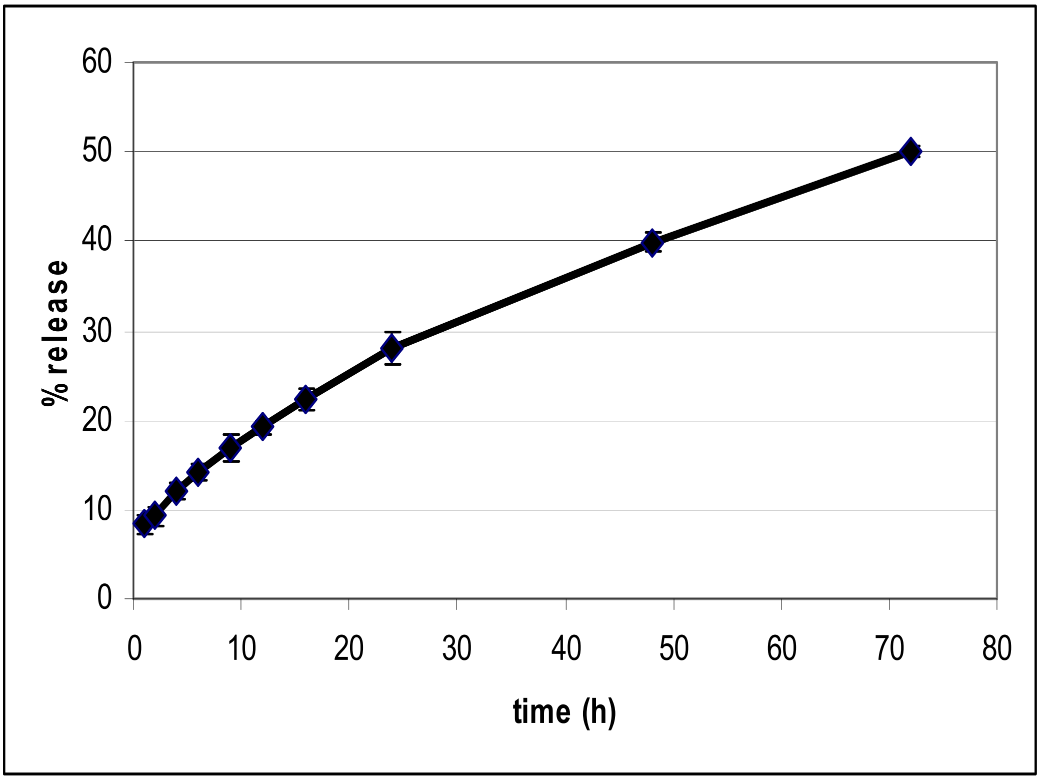


Figure 6. Mean ***in vitro*** **release test results for product A using the basket method at 100 rpm with vertical error bars showing one standard deviation on each side**

First order kinetics : $\ln(1 - F) = -k_1t$ (2)

where F represents the fraction of drug released in time t and k_1 is the first order release rate constant.

Higuchi model : $F = k_2t^{1/2}$ (3)

where F represents the fraction of drug released in time t and k_2 is the Higuchi dissolution constant.

Hixson–Crowell model : $W^{1/3}{}_0 - W^{1/3}{}_t = k_3\ t$ (4)

where W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and k_3 is a constant .

Korsmeyer–Peppas model : $F = k_4t^n$ (5)

where k_4 is a rate constant, and n is the release exponent.

Table 7 shows the results of the fitting to the above models.

Of all the above-mentioned models, only the first order release model gave r^2 values greater than 0.95 for all the formulations. The dissolution profiles were also compared by the f_1 and f_2 difference and similarity tests described in the FDA guidance for dissolution testing of immediate release solid dosage forms (FDA, 2000).

$$f_1 = \{[\sum_{t=1}^{n} |R_t - T_t|]/[\sum_{t=1}^{n} R_t]\} \times 100 \quad (6)$$

$$f_2 = 50 \times \log \{[1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \quad (7)$$

Table 7. Fitting the release profiles of the three products

Product	Release model										
	Zero order		First order		Higuchi		Hixson-Crowell's		Korsmeyer-Peppas		
	release rate constant (min^{-1})	r^2	release rate constant (min^{-1})	r^2	release rate constant ($min^{-0.5}$)	r^2	release rate constant ($mg^{1/3}.min^{-1}$)	r^2	release rate constant (min^{-n})	release exponent	r^2
A	0.009139	0.907	0.026	0.9852	0.12738	0.9612	0.0444	0.968	2.3456	0.8469	0.9454
B	0.010761	0.7738	0.032	0.9526	0.15379	0.8671	0.0530	0.9026	0.4889	1.2647	0.8469
C	0.010192	0.7727	0.033	0.9557	0.14599	0.8699	0.0525	0.9084	0.7894	1.1542	0.7699

The R_t is the %release of the reference product at the time t and the T_t is the %release of the test product at the time t, while n is the number of data points of the dissolution profile (FDA,2000).

Both factors are model-independent measures of the similarity of two multipoint dissolution profiles (FDA, 2000). Two products are considered to have similar dissolution profiles when f_1 is not greater than 15 and f_2 is greater than 50 (FDA, 2000).

When applied to the dissolution profiles of the three products, these tests showed the three products to be similar to each other as shown in table 8.

Table 8. Results of calculating the similarity and difference factors for the release profiles of the three products.

Reference	Test	f_1	f_2
A	**B**	**14.8**	**54**
A	**C**	**13.9**	**54.7**
B	**C**	**5.7**	**66.7**
C	**B**	**5.3**	**66.7**

Cosolvents are generally not desirable for use in dissolution media (Gowthamarajan and Singh, 2010), and, since they can interact with the suppository base, they were not used. HPBCD was used at 10% w/w concentration at which sink conditions are certainly achieved as they have been shown to be achieved at even lower HPBCD levels. The paddle rotation speed employed was 100 rpm since at 50 and 75 rpm the %RSD values obtained exceeded 20% at more than 30% release.

Other configurations were also attempted but were not successful. Basket method at 100 rpm gave 50% release after 72 hours while the dialysis cell gave even slower release rendering both procedures impractical, and, anyway, procedures involving the use of dialysis membranes are not recommended as they introduce an artificial step of the drug molecules crossing the dialysis membrane to the release process (Siewert *et al.*, 2003). The excessively slow release observed in the basket method can be attributed to the fact that, after melting, the suppository remains entrapped within the basket without spreading resulting in a thick mass of fat with little surface area, whilst in the paddle apparatus, the molten suppository spread over the surface of the dissolution medium. The very slow release observed in the dialysis cell experiment can be also attributed to the limited surface area available for drug release, in addition to the possible additional slowing down of the release due to the presence of the additional step of the drug crossing the dialysis membrane, and to the dialysis cell impeding any agitation of the molten suppository inside it and of the suppository's immediate environment.

Since both progesterone and fatty suppository bases are neutral substances the properties of which are not affected by pH, there was no need to use buffers. As for the relatively high relative standard deviation values obtained, they are not unusual for suppositories (Itiola and Adegboye, 2008). It is, probably, for this reason that the FDA accepted a dissolution testing method for miconazole nitrate pessaries that gave high RSD values even reaching, at a point a value of 24.0% at 40% release (FDA, 1999).

3.4-*In vivo* pharmacokinetic studies results

The mean pharmacokinetic profiles obtained are shown in figure 7.

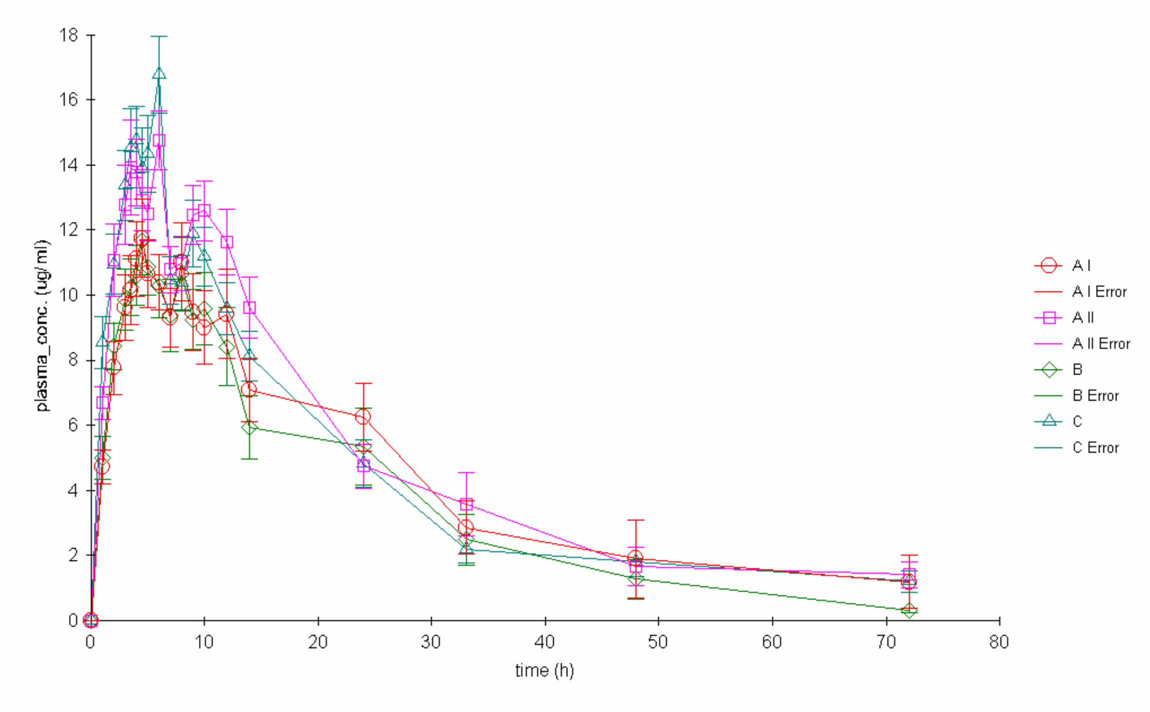


Figure 7. Mean pharmacokinetic (PK) profiles obtained during the *in vivo* PK studies with vertical error bars showing one standard error on each side

Since two pharmacokinetic studies were performed on the product A, two pharmacokinetic profiles are shown for it: A I for the data obtained from the study comparing the product B with product A, and A II for the data obtained from the study comparing product C against product A.

Non-compartmental analysis results for the four PK profiles are shown in table 9. Both test formulations were shown to be bioequivalent to the reference formulation. The two test formulations will, on the first glance seem bioinequivalent; however, the differences between them are due to them being tested on different occasions, as is shown by the fact that similar differences exist between the results of the reference product in the two studies.

As for compartmental analysis, it was used to estimate the absorption and elimination rate constants (k_a and k_e respectively) shown in table 10. The Akaike Information Criterion (AIC) was better for the one compartment model for the majority of the subjects, and, thus, one compartment model was applied. Since progesterone is known to exhibit a considerably short elimination half-life (Drug Information Handbook, 2008), the PK profiles exhibit flip-flop behavior.

Table 9. Results of non-compartmental analysis of PK data

		A I	A II	B	C
Tmax (h)	**Mean**	**7.27**	**6.56**	**6.71**	**5.88**
	SD	**7.94**	**5.58**	**4.47**	**2.86**
Cmax (µg/ml)*	**Mean**	**12.70**	**19.40**	**13.87**	**19.53**
	SD	**1.60**	**1.49**	**1.72**	**1.40**
AUC_{last} (µg.h/ml)*	**Mean**	**209.9**	**264.6**	**206.1**	**252.5**
	SD	**2.06**	**1.56**	**2.03**	**1.57**
AUC_0^{∞} (µg.h/ml)*	**Mean**	**213.1**	**296.2**	**211.7**	**286.7**
	SD	**1.84**	**1.62**	**2.01**	**1.53**
MRT (h)#	**Mean**	**18.97**	**16.40**	**17.15**	**15.85**
	SD	**59.7**	**41.8**	**63.3**	**36.6**

***geometric mean and SD**
#harmonic mean and SD

Table 10. Mean±SD values of k_a and k_e values obtained by compartmental analysis

	A I	A II	B	C
k_a (h^{-1})	**0.0915±0.0676**	**0.0731±0.0412**	**0.109±0.0790**	**0.0832±0.0506**
k_e (h^{-1})	**0.495±0.345**	**0.672±0.975**	**0.651±0.996**	**0.751±0.647**

A deconvolution procedure was performed on the PK profiles using a mono-exponential unit impulse response (UIR) obtained from compartmental analysis to obtain fraction absorbed versus time profiles that were used to construct Levy plots. The two absorption profiles generated for product A were averaged together. Figure 8 shows the resulting absorption profiles, which are clearly similar.

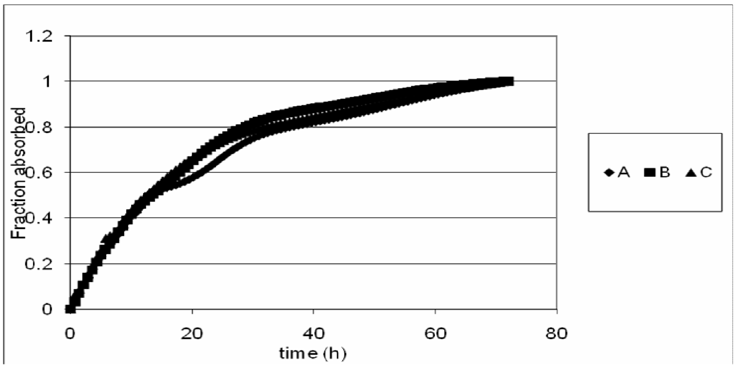


Figure 8. Absorption profiles for the three products obtained by deconvolution

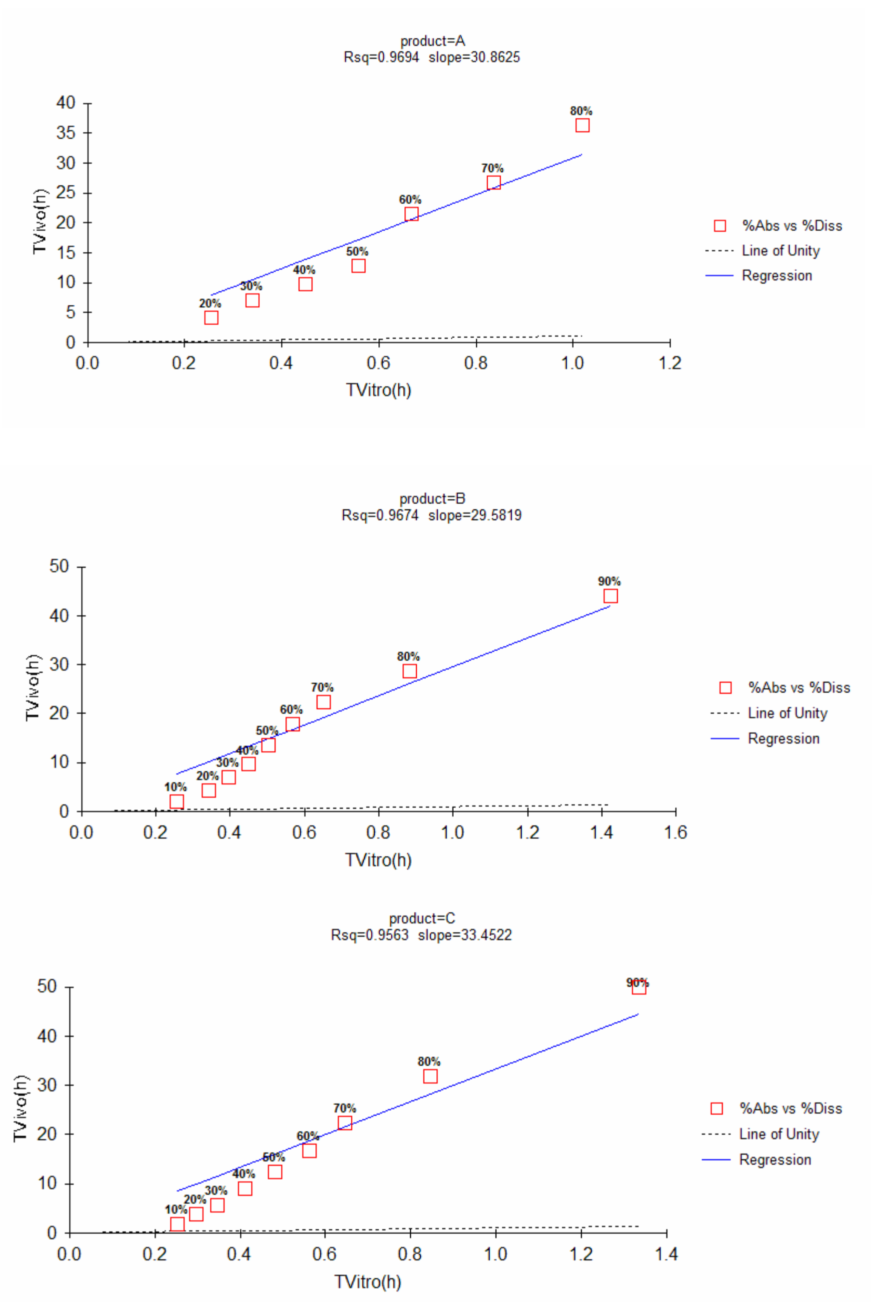


Figure 9. Levy plots for the products A (top), B (middle) and C (bottom)

The resulting Levy plots exhibit high correlation coefficients (r^2>0.95) and similar slopes indicating a strong potential for IVIVC, and that the method is potentially biorelevant; however, the lack of products with significantly different release rates precludes validating an IVIVC and checking the discriminating power of the method.

4-Conclusion

Despite their apparent simplicity, progesterone vaginal suppositories are a challenging dosage form in terms of developing a suitable *in vitro* release testing procedure due to the variability in their behavior. HPBCD can be used to provide sink conditions for the release of progesterone from the pessaries as it is capable of sufficiently solubilizing the drug without dispersing the base and so providing gentle conditions unlike the use of extremely high concentrations of surfactants. Our method, employing a 10% w/w HPBCD solution as the dissolution medium using USP type II dissolution testing apparatus at 37^{o}C at paddle rotation speed of 100 rpm, showed good potential of being biorelevant. However, *in vitro* and *in vivo* evaluation of products with significantly different release rates is required to validate an IVIVC and check its discriminating power.

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تطوير ظروف اختبار انحلال ملائم حيوياً لتحاميل مهبلية للبروجستيرون دهنية القاعدة و إمكانية استخدامها لبناء ارتباط لتصرف الانحلال خارج الجسم و داخله

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الملخص

هدف هذه الدراسة هو تطوير إختبار إنحلال ملائم لتحاميل مهبلية دهنية القاعدة للبروجستيرون بهدف الحصول على فحص رقابة نوعية مناسب و دراسة قابلية هذا الإختبار للاستخدام للحصول على ارتباط لتصرف الإنحلال خارج الجسم و داخله. تم استخدام كل من هيدروكسيبروبيلميثيل-بيتا-سيكلودكسترين و دوديسيل كبريتات الصوديوم لإذابة البروجستيرون للحصول على ظروف البالوعة. و لكن التركيز اللازم من دوديسيل كبريتات الصوديوم كان شديد الارتفاع و وفر بيئة قاسية للإنحلال فتم استخدام محلول يحتوي على تركيز 10% من الهيدروكسيبروبيل-بيتا-سيكلودكسترين من وزنه و تم فحص استخدامه في ظروف مختلفة. استخدام 900 مل من المحلول المذكور في جهاز فحص الانحلال من طراز رقم اثنين حسب دستور أدوية الولايات المتحدة على حرارة 37 درجة مئوية و سرعة دوران المرساة 100 دورة للدقيقة أعطى نتائج مقبولة. بيانات الإنحلال وبيانات تراكيز الدواء في البلازما تم الحصول عليها من نتائج دراسات تكافؤ حيوي لثلاث منتجات مختلفة استخدمت للحصول على رسوم ليفي, و قد أعطت هذه الرسوم معامل ارتباط أعلى من 0,95 مما يدل على وجود إمكانية جيدة للحصول على ارتباط لتصرف الإنحلال داخل الجسم و خارجه, و لكن للتثبت من ذلك و من القدرة التفريقية لإختبار الإنحلال يبقى من اللازم إجراء الدراسات المذكورة على تركيبات ذات معدلات انحلال مختلفة جدياً عن التركيبات المذكورة.